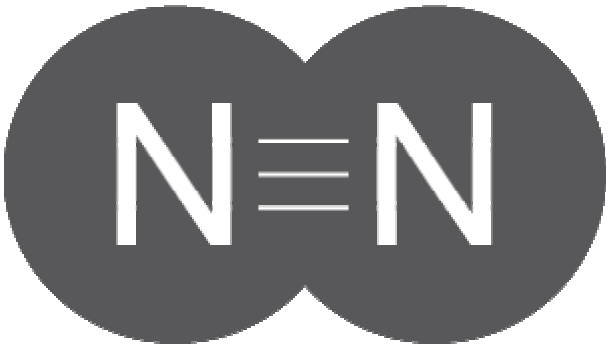

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PS3-3 Role of the PAS2 domain of NifL in signal transduction

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The NifL-NifA system regulates the transcription of genes required for nitrogen fixation in *Azotobacter vinelandii*. The activity of the transcriptional activator, NifA, is controlled by its partner protein NifL, in response to changes in redox potential and fixed nitrogen status. The ability of NifL to inhibit transcriptional activation by NifA is mediated solely through the formation of the NifL-NifA protein-protein complex. NifL contains two N-terminal PAS domains and a C-terminal region similar to histidine protein kinases. The first PAS domain, PAS1, contains a FAD co-factor and is responsible for redox sensing, whereas the second PAS domain, PAS2, has no known co-factor and its function remains unclear. We have identified two classes of mutation in the PAS2 domain of NifL. The first class results in a "locked-on" form of NifL that constitutively inhibits NifA, irrespective of environmental conditions. The second class of mutation results in failure to transduce the redox signal so that the mutant proteins fail to inhibit NifA under oxidising conditions, but retain the capacity to respond to the nitrogen status. These results suggest that PAS2 plays a pivotal role in transducing the redox signal from PAS1 to the C-terminal domains of NifL. Using the bacterial adenylate cyclase two-hybrid (BACTH) system, we have detected an interaction between subunits of the isolated PAS2 domain, suggesting that this domain is a multimer. Biochemical studies demonstrate that the isolated PAS2 domain is dimeric in solution. The PAS2-PAS2 interaction detected by BACTH is maintained in PAS2 mutations that give rise to a defective redox response, but is perturbed by mutations in PAS2 that result in a "locked-on" phenotype. This suggests a model for signal transduction in NifL whereby redox-dependent conformational changes in PAS1 are relayed to the C-terminal domains of NifL via changes in the quartenary stucture of the PAS2 domain.